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Isolation of Fungal Pathogens from Scalp Infection and Its Control using Herbal Products

R. Muthulakshmi

Department of Microbiology, AVC College (Autonomous),
Mannampandal – Mayiladuthurai – Nagappattinam Dist., India.

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Fungi is a major problem to cause diseases on the skin and scalp The most frequent and abundant species were: *Cladosporium cladosporioides*, *Cl. herbarum*, *Penicillium chrysogenum* and *Aspergillus flavus*. *Microsporum canis*, *Aphanoascus fulvescence* and *Chrysosporum sulfureum* were the most frequent and abundant species of all dermatophytes and dermatophyte-like keratinophilic fungi recovered. In the current study an attempt is made in isolating the fungi from scalp region and it was grow in SDA medium. The isolated colonies were identified by using lactophenol cotton blue staining. The fungi identified and controlled by using two herbal products. First , the efficiency of the neem oil and lemon extract in different concentrations such as 5%,10%,15% and 20% were used to control the fungi by using carborundum method. Second , the efficiency of fungi was tested using neem oil and lemon extract in different concentrations such as .5ml,1ml and 2ml by using minimum inhibitory concentration method.

Key words: Fungal pathogens, Scalp infection, Herbal products.

In humans, the scalp is a specialized area of skin on top of the head, usually covered in both sexes. The scalp contains as many as 150,000 hair follicles. It is usually described as having five layers, which can be remembered with the mnemonic 'scalp' [Hiddell and scott, 1889]. S-the skin on the head from which head hair grows. It is richly supplied with blood vessels. C-connective tissue , a thin layer of fat and fibrous tissue lies beneath the skin . A-aponeurosis [or galea aponeurotica] is the next layer . It is tough layer of dense fibrous tissue which runs from the frontalis muscle anteriorly to the occipitalis posteriorly.

L-the loose areolar connective tissue layer provides an easy plane of separation between the upper three layer and the pericranium. In scalping, the scalp is torn off through this layer. It also provides a plane of access in craniofacial surgery and neurosurgery . This layer is sometimes referred to as the danger zone. Because of the ease by which infectious agents can spread through it to emissary veins which then dry into the cranium. P-the pericranium is the periostem of the skull bones and provides nutrition to the bone and the capacity of repair. It may be lifted from the bone to allow removal of bone windows.

The clinically important layer is the aponeurosis. Scalp lacerations through this layer mean that the anchoring of the superficial layer is lost and gaping of the wound occurs; this requires suturing. This can be achieved with simple or vertical matress sutures using a non absorbable material, which are subsequently removed at around day seven. Scalp conditions : Scalp itchiness is a problem afflicting 43 percent of the general population atleast once in 12- month period. Many factors contribute to this including blow –dryers, styling products; hats, allergies, dandruff, mites and even stress. The scalp can reflect the overall conditions of the body and is affected by stress and hormonal changes [Norman and William,1934]. Dandruff is a common problem

* To whom all correspondence should be addressed.
e-mail: vijayachinns@gmail.com

due to the excessive shedding of dead skin cells from the scalp. Cutis verticisggrata is a descriptive term for a rare deformity of a scalp. Psoriasis is a scalp condition where hyper-proliferation of cells causes them to bunch up at the suface , taking in the form of large, thick, white scales and sheets of skin that actually slough off in these larger sheets (Amy stanway, 2008).

***Azadirachta indica* (Neem)**

Neem tree of family meliaceae is evergreen tree found in most tropical countries. Almost every part of the tree has been in use since ancient times to treat a number of human ailments and also as a household pesticide. The extract from bark, leaves, fruits and root have been used to control leprosy, intestinal helminthiasis and respiratory disorders in children. The bark extract is also used as tonic, astringent and useful in relieving fever, thirst, nausea, vomiting and skin disease. The immunomodulatory activity of the neem – bark extract has also been reported. The medicinal and industrial uses of various parts of neem tree and the compounds isolated have been reviewed. The bark powder contains sugar, proteins, aminoacid and oil.polysaccharides such as arabinafucoglucanes and fucogalactogluco-arabinanes have also been isolated from neem bark. Flavonoids, flavonoglycosides, dihydro-chalocones, tannins and others are also important constituents of bark, leaves, fruits and flowers of neem. The biological activities and medicinal properties of neem have recently been reported. In the present study, we report in vitro study of potential of neem oil to inhibit fungal activities (Wafaa *et al*, 2007).

***Citrus limon* (lemon)**

The lemon is a hybrid in cultivated wild plants. It is the common name for the reproductive tissue surrounding the seed of the angiosperm lemon tree. The lemon is used for culinary and non culinary uses throughout the world. The fruit is used primarily for its juice through the pulp and rind (zest) are also used, primarily in cooking and baking. Lemon juice is about 5% citric acid, which gives lemons a tart taste and a pH of 2 to 3. This makes lemon juice an inexpensive, readily available acid for use in educational science experiments.

Medicinal uses

Lemon juice is widely known as a diuretic antiscorbutic , astringent and febrifuge. In Italy, the sweetened juice is given to relieve gingivitis, stomatitis and inflammation of the tongue. Lemon juice in hot water has been widely advocated as a daily laxative and preventive of the common cold, but daily doses have been found to erode the enamel of the teeth. (Julia 1999).

Review of literature

Ajello *et al.,* (1966) cultured the skin scrapings into sabouraud dextrose chloramphenicol actidione agar. A duplicate inoculation of the specimen was also cultured on sabouraud dextrose cycloheximide agar. The plates were incubated at 28°C for up to 4 weeks and examined at 2-3 day intervals for fungal growth. Robbins (1976) have been shown *citrus spp*. Peels have the capacity to reduce erythrocyte aggregation and sedimentation rates in human blood , as well as to exibit antiviral, antimutagenic and antimicrobial properties. Rebell *et al.,* (1979) reveals that fungal infections of the scalp can be effectively treated with oral antifungal drugs and good hair care. The source of infection e.g. pets should be treated simultaneously to prevent recurrents. Early recognition and treatment of the fungal infection will prevent permanent scaling and balding. Longbottom (1983) examined that seborrheic dermatitis of the scalp severe enough to result in hair loss is generally accompanied by seborrheic dermatitis of the retroauricular and nasal folds. This, in turn, is followed by worsening of the underlying disease and accumulation of loose telogen hairs, which are then shed when the scalp is once again finally shampooed. De vrocy (1985) conducted a test to the study of scalp infection. He recognise that scalp infection is caused by a combination of fungi and bacteria. It causes scaling and sogginess of the scalp, commonly of the hair shaft region. Sometimes the scalps becomes pale and can be itchy. The infection is often picked up from contaminated skin fragments in public places, such as swimming pools and shower facilities.

Lenette *et al*., (1985) performed an experiment that an inoculum of each of the fungal strain was suspended in 2 ml of sabouraud dextrose broth and incubated at 25-2°C for 4-5 days. They observed zones of inhibition which is caused by the efficiency of *A. indica.* Afek *et al.* (1986) gave the results that citrus species are

components within the fine structure of human scalp hair shafts

Ali-Shtayeh *et. al* (2001) reveals that hair and scalp mycobiota of 1369 clinically normal children aged 6-12 years attending 12 schools in the nablus Distric , using the hair brush technique. One hundred and one fungal species belonging to 33 genera were recovered. Species varied considerably in their frequency of occurrence and abundance based on their relative importance values (RIVs). Mc Neil *et al.,* (2001) demonstrate that the number of life-threatening , invasive fungal infections has risen dramatically over the last 20 years. Totally 4% of all patients dying in modern tertiary care hospitals have *Aspergillus*, 2% of *Candida.* Currens *et al.,* (2002) performs a study in scalp itching. It is a problem affecting 43% of the general population at least once in 12 months period. Many factors contribute to this including blow dryers, styling products, allergies, dandruff, mites and even Stress. The scalp can reflect the overall conditions of the body and is affected by stress and hormonal changes

MATERIAL AND METHODS

Isolation of fungi from scalp

Collection of sample

The sample was collected by using sterilized cotton swabs. The sterile swab was gently rotated on the scalp region. Sterile condition was maintained during the sample collection. Then the swab was used for culturing process.

Isolation

1. Sabouraud dextrose agar medium (SDA) was prepared and sterilized in the autoclave at 121°C for 15 lb pressure for 15 minutes. Then the medium was allowed to cool.
2. 0.5g of streptomycin was added to the sabouraud dextrose agar medium and mixed well. The cooled medium was poured into the sterile petriplates. It was allowed to solidify.
3. After solidification, the swab was lawned on the surface of sabouraud dextrose agar medium. Then the plates were incubated at 25-28°C for 3-6 days.
4. After incubation, pure culture was obtained and maintained by inoculating the colonies on sabouraud dextrose agar medium.

Identification

Staining technique

1. The fungi which was Isolated in the cultering process was observed under microscope by using lactophenol cotton blue staining.
2. Clean grease free slide was preferred for this staining and it was washed in the tap water.
3. One drop of lactophenol cotton blue stain was placed on the sterile slide.
4. Sterile inoculation needle was used for the pick up the fungal mycelium and it was mixed with the dye.
5. Finally coverslip was placed over the slide and care should be taken to avoid air bubbles and it was examined under microscope (low and high power objectives).

Antifungal assay

Antifungal assay was identified by using two herbal products Neemoil and lemon extract.

Preparation of neem oil concentration

Commercially available neem oil was used for antifungal assay. Different concentration of neem oil used in this technique was 5%,10%,15%,20%.

Preparation of lemon extract

Lemon extract was prepared by filtering the lemon juice using whatman filter paper and it was used for antifungal assay. This extract was make up to different concentration such as 5%,10%,15%, and 20%.

Procedure for antifungal activity

Prepare SDA medium and it was sterilized in the autoclave at 121°C for 15lb pressure for 15 minutes. It was allowed to cool for few minutes. 0.5gm of streptomycin was added to the SDA medium and mixed well. The different concentration of neem oil and lemon extract were added to the medium separately and mixed well. The control plate was maintained without the concentration of neem oil and lemon extract. The medium containing different concentration of neem oil and lemon extract was poured into the sterile petriplates and allowed to solidify. The identified fungi was inoculated with the help of carborundum. The inoculated plates were incubated at 25-28°C for 3-6 days.

Minimum inhibitory concentration of neem oil and lemon extract

Sabouraud dextrose broth was prepared and small pinch of streptomycin was added to the medium. The different concentration of neem oil and lemon extract (.5ml,1ml and 2ml) was added to each tubes. Then the fungal spores were inoculated into the broth containing different concentration of herbal product.the tubes were incubated at 25-28°C for 3-6 days. After incubation the tubes were examined for spectroscopic assay.

RESULTS AND DISCUSSION

Isolation

After incubation , the plates were observed for the presence of colonies. On SDA medicine different types of fungi were isolated from the scalp region and it was identified by using fungi manual. Depending upon the size, color and texture of the colony, the fungi was identified as *Aspergillus fumigatus, A.Niger* and *Cadida albicans.*

On SDA medium *Aspergillus fumigatus* produce blue-green to dark green colonies which are powdery in nature.

On SDA medium *Aspergillus niger* shows carbon black to very dark brown spores which are rough in nature.

Colonies on SDA medium at 25°C, *Cadida albicans* produce white to cream, soft and smooth wrinkled and Nuecilagenous. But the isolated colonies were confirmed by using staining techniques

Identification

Using lactophenol cotton blue staining , the isolated colonies such as *Aspergillus fumigatus*, *Aspergillus niger* and *Candida albicans* were confirmed

Antifungal activity

Effect of Neem oil on the Growth of the Test Organisms

Growth rate of the test organisms were inhibited by neemoil and test organisms were inculcated at various concentration of neemoil (5,10,15 and 20 %) and incubated for 3 days . The main growth rate of the test organism *Aspergillus Niger, Aspergillus fumigatus , Candida albicans* were progressively decreases with increase in the concentration of neemoil on SDA medium when compared to control. (Table 1).

The radial growth was measured periodically and the mean growth rate was calculated. The percentage inhibition of growth was calculated as follows

$$\text{Percentage inhibition of growth} = \frac{\text{Growth in Control - Growth in Treatment}}{\text{Growth in Control}}$$

Effect of Lemon extract on the growth of the organism

The growth rate of the test organisms were suppressed by lemon extract and test organisms were inculcated at different concentration of lemon extract (5,10,15 and 20 %) and allowed to incubate for 3 days. The mean growth rate of *Aspergillus niger, Aspergillus fumigatus* and *Candida albicans* were progressively decreasing with increase in the concentration of lemon extract on the SDA medium when compared with control. (Table 2 , Plate 4b, 5b, and 6b). The radial growth was measured periodically and the mean growth rate was calculated. The percentage inhibition of growth was calculates as follows

$$\text{Percentage inhibition of growth} = \frac{\text{Growth in Control - Growth in Treatment}}{\text{Growth in Control}} \times 100$$

Analysis of MIC

The MIC was determined as the lowest concentration of neemoil and lemon extract that inhibited the growth of the organisms such as *Aspergillus niger, Aspergillus fumigatus* and *Candida albicans*. Different concentrations of neemoil and lemon extract such as 0.5 ml , 1ml and 2 ml were tested against the tree fungi. Finally the MIC were determined for all three fungi

CONCLUSION

Fungi is a major problem to cause diseases on the skin and scalp. In the current study an attempt of isolating fungi from scalp region and it was grow in SDA medium. The isolated colonies were identified by using lactophenol cotton blue staining. The fungi identified and controlled by using two herbal products. First , the efficiency of the neem oil and lemon extract in different concentrations such as 5%,10%,15% and 20% were used to control the fungi by using carborundum method. Second, the efficiency of fungi was tested using neem oil and lemon extract in different concentrations such as .5ml,1ml and

2ml by using minimum inhibitory concentration method. From the above findings it was inferred that fungi isolated from the scalp region were controlled by using the two herbal products(neem oil and lemon extract)

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